

**Patent claims**

1. Method for confocal 4-pi microscopy, characterized by the following steps:

- 5 • Coherent illumination of a sample from two sides by one objective each with illumination light that has at least one illumination wavelength, whereby a stationary illumination wave with one main illumination maximum and with secondary illumination maxima is produced by interference of the illumination light in the sample, and
- 10 • Detection of the detection light emitted by the sample that exhibits at least one detection wavelength, that passes through the two objectives, whereby the detection light is made to interfere, thereby producing in the sample a detection pattern with one main detection maximum and with secondary detection maxima produced such that the secondary illumination maxima
- 15 and the secondary detection maxima are located in different places.

2. Method according claim 1, wherein the objectives each exhibit a pupil, and wherein the spatial position of the secondary illumination maxima and/or the secondary detection maxima can be set and/or changed by introduction of pupil filters on at least one pupil plane or on at least one plane

20 that corresponds optically to the pupil plane.

3. Method according to one of claims 1 or 2, wherein the sample is marked with at least one fluorescent dye.

4. Method according to claim 3, wherein excitation of the sample is accomplished by multiple-photon excitation, particularly by two-photon

25 excitation.

5. Method according to claim 3, wherein excitation of the sample comprises a Foerster-resonant energy transfer (FRET) within the sample.

6. Method according to claim 3, wherein the illumination light exhibits a further illumination wavelength, and occurs via a virtual or via a real  
5 intermediate level.

7. Method according to claim 3, wherein excitation occurs via a higher excitation level, in particular via an  $S_0$ - $S_2$  transition.

8. Method according to one of claims 1 to 7, wherein the illumination wavelength to detection wavelength ratio is in a range of 0.5 to 0.9, in particular  
10 in a range of 0.6 to 0.8, in particular 0.75.

9. Method according to one of claims 3 to 8, wherein the fluorescent dye exhibits an excitation and an emission region, whereby the illumination wavelength is selected from the high-frequency portion of the excitation region and/or from the low-frequency portion of the emission region.

10. Method according to one of claims 1 to 9, wherein a detection pinhole aperture is envisaged, whose aperture diameter is set smaller than 1 Airy disc, in particular 0.7 to 0.8 Airy discs, in particular 0.7 Airy discs.

11. Confocal 4-pi microscope with a light source that generates an illumination light that exhibits at least one illumination wavelength, and that is  
20 directable from two sides by one objective each onto a sample, in which a stationary illumination wave having a main illumination maximum and secondary illumination maxima is produced by interference of the illumination light in the sample, and with a detector to detect detection light emitted by the

sample that passes through both objectives and that exhibits at least one detection wavelength, characterized in that the detection light interferes and a detection pattern is produced in the sample with a main detection maximum and with secondary detection maxima, whereby the secondary illumination maxima and the secondary detection maxima are located at different places.

12. Confocal 4-pi microscope according to claim 11, wherein the objectives each exhibit one pupil and wherein pupil filters can be introduced on at least one pupil level or on at least one level corresponding optically to the pupil level, which establish the spatial position of the secondary illumination maxima and/or of the secondary detection maxima.

13. Confocal 4-pi microscope according to claim 12, wherein the minimum of one pupil filter exhibits a phase plate that comprises in particular regions of varying phase delay, in particular a  $\lambda/2$  plate.

14. Confocal 4-pi microscope according to one of claims 11 to 13, wherein the minimum of one detection wavelength is selectable and the detector may be set to the selected detection wavelength.

15. Confocal 4-pi microscope according to one of claims of 11 to 14, wherein the minimum of one illumination wavelength is selectable, and the light source can be set to the selected illumination wavelength.

16. Confocal 4-pi microscope according to one of claims 11 to 15, wherein the sample is marked with at least one fluorescent dye.

17. Confocal 4-pi microscope according to claim 16, wherein the light source is a laser, particularly a pulse laser, and wherein excitation of the sample is achieved by multiple-photon excitation, particularly by two-photon excitation.

18. Confocal 4-pi microscope according to claim 16, wherein excitation of the sample comprises Foerster-resonant energy transfer (FRET) within the sample.

19. Confocal 4-pi microscope according to claim 16, wherein the illumination light exhibits a further illumination wavelength, and excitation of the sample occurs via a virtual or via a real intermediate level.

20. Confocal 4-pi microscope according to one of claims 16 to 19, wherein excitation occurs via a higher excitation level, particularly via an  $S_0$ - $S_2$  transition.

21. Confocal 4-pi microscope according to one of claims 11 to 20, wherein the illumination wavelength to detection wavelength ratio lies in a range between 0.5 to 0.9, in particular in a between range 0.6 to 0.8, in particular 0.75.

22. Confocal 4-pi microscope according to one of claims 16 to 21, wherein the fluorescent dye exhibits an excitation and an emission region, whereby the illumination wavelength lies within the high-frequency portion of the excitation region and/or the detection wavelength within the low-frequency portion of the emission region.

23. Confocal 4-pi microscope according to one of claims 11 to 22, wherein the confocal 4-pi microscope exhibits a detection pinhole aperture having an aperture diameter smaller than 1 Airy disc, in particular 0.7 to 0.8 Airy discs, in particular 0.7 Airy discs.